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**(54) Neutral buoyance glass-surface
microcarrier for growth of cell
cultures, and method of
manufacture**

(57) A microcarrier for anchorage-
dependent cell culturation comprising,
and preferably consisting essentially
of, a spherical substrate of polymeric
material having a bulk density of
about 1 g/cc so as to be substantially

buoyant in an aqueous culture
medium, and a thin (less than 1 μ m)
coating layer of silicate glass. The
silicate glass coating layer is
preferably applied to a polymeric
precursor in an rf sputtering operation.
An intermediate coating layer of
magnetic material may be deposited
prior to the silicate glass layer, so that
the microcarriers may be readily
removed from culture media by
suitable subjection to a magnetic field.

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SPECIFICATION

Neutral buoyancy glass-surface microcarrier for growth of cell cultures, and method of manufacture

5 The present invention relates to microcarriers for growth of anchorage-dependent cell cultures. More particularly, the invention relates to glass-surface microspheres specifically adapted for use as such microcarriers, and to methods for

10 manufacture of such microspheres.

In the art of growing anchorage-dependent cell tissue cultures, it has heretofore been proposed to replace the standard roller bottles and petrie dishes with so-called microcarriers for providing

15 enhanced surface area for cell attachment. The United States patent to Levine et al. 4,189,534 proposes, for example, that microcarriers in the form of solid plastic beads be employed. It has been found, however, that plastic microcarriers of this type require alteration of electrically charged surface moieties to promote cell attachment, which alteration is difficult to control

20 quantitatively in production and is toxic to some types of cell cultures if not properly controlled. It is also difficult to remove some cell types from the plastic bead surface.

It has also been proposed to employ solid glass beads as cell microcarriers. In addition to the aforementioned problems, a significant

30 disadvantage of microcarriers previously proposed, including specifically solid beads of plastic or glass, is a difficulty in controlling or tailoring the density of the microcarrier to that of the selected culture medium. Conventional cell culture media are aqueous in nature and possess densities generally in the range of 1.03 to 1.09 g/cc. Silica glass beads, which possess desirable surface qualities, typically have a density on the order of 2.3 g/cc depending upon glass composition. To avoid settling and compaction of the microcarriers in the growth medium, which tends to inhibit cell growth, it is necessary to stir or otherwise continuously agitate the culture medium. However, vigorous agitation is itself

45 destructive to many cell types. The art relating to microcarriers for animal cell cultures in general is reviewed in 3rd General Meeting of ESACT, Oxford 1979, *Develop. Biol. Standard*, 46, pp. 109—294 (S. Karger, Basel 1980).

50 In the copending U.S. application of Downs et al., Serial No. 332,377, filed December 21, 1981 and assigned to the assignee hereof, the foregoing and other difficulties in the art are addressed by forming hollow glass precursor microspheres of silicate glass composition, and then tailoring the density of such precursor microspheres in a post-forming etching operation to match closely the density of the desired aqueous growth medium. This technique has proven successful in

60 overcoming both the surface-charge and the buoyancy problems of the earlier art. However, the number of separate operations involved makes cost reduction desirable.

A general object of the present invention,

65 therefore, is to provide a microcarrier having a density which closely matches that of typical cell culture media and embodies the desirable surface characteristics of silicate glass, but is less expensive to manufacture than are microcarriers previously proposed which embody similar benefits.

Another object of the present invention is to provide a microcarrier which may be readily removed from the culture media, and which is particularly well adapted to rapid removal using automated processes.

Another object of the invention is to provide a method of manufacturing such microcarriers.

Briefly states, microcarriers are manufactured

80 in accordance with one important aspect of the present invention by first fabricating a spherical precursor of polymeric material having a precursor bulk density substantially the same as that of the desired aqueous growth medium (about 1 g/cc), and then coating the polymeric precursor with a thin layer of silicate glass. The thin glass layer does not significantly alter the effective bulk density of the precursor, while imparting thereto the significant advantages of a glass attachment surface in terms of toxicity and ease with which cultures may be removed therefrom without significant damage. The microcarrier in accordance with the invention therefore comprises, and preferably consists essentially of, a

90 neutral-buoyancy (about 1 g/cc) spherical polymeric precursor and a thin (no greater than 1 μ m) continuous surface coating of silicate glass.

In accordance with another important aspect of the present invention, microcarriers are provided with a composition consisting in part of magnetic material, whereby the microcarriers may be attracted by a magnetic field and readily removed from the culture medium. Preferably, such magnetic material comprises a thin coating of magnetic material deposited on the polymeric precursor in the foregoing discussion prior to deposition of the outer layer of silicate glass.

The spherical polymeric precursor may be formed by any conventional technique. For

110 example, a film-forming polymer may be dissolved in a suitable solvent and then sprayed into the upper portion of a heated chamber or furnace in the form of atomized droplets. As the droplets fall by gravity within the heated chamber, the solvent rapidly evaporates and a polymeric skin or shell is formed. The U.S. patent to Veatch et al. 2,797,201 discloses such a process and an apparatus for practicing such process. Nominal precursor size and density are controlled as a

120 function of polymer/solvent concentration, drying temperature and droplet size. Droplets may typically be in the range of 5 to 500 microns in size. Drying temperatures may be in the range of 20°C to 500°C. The produce precursor may be collected dry at the bottom of the chamber, cleaned and sieve cut to the desired size range, such as the range of 106 to 200 microns.

The polymeric precursor may also be formed as solid beads by spraying and cooling molten

polymers, directing a droplet generator or other conventional apparatus into a free-fall zone such that the droplets are cooled and solidified during free-fall and collected. Alternatively, solid pre-sized pieces (frit) of polymeric material may be fed into a tower furnace for melting and reforming as spherical beads during free-fall.

The polymeric precursor material is selected to yield the desired bulk density at the desired size range, and for glass coatability. Polystyrene and polyethylene are examples of suitable materials. The precursor may be solid, hollow or porous. Depending upon the precursor material and the coating method to be employed, it may be desirable to surface-treat the precursor so as to render the same more susceptible to silicate glass coating. Precursor diameter in the range of 50 μm to 500 μm and density in the range of 1.00 to 1.10 g/cc are preferred.

Silicate glass coating of the polymeric precursor is preferably accomplished by rf sputter deposition. (The term "silicate glass" as used herein refers to a glass which includes oxides of silicon, with or without other metallic oxides.)

Most preferably, a magnetron rf sputtering unit is employed to obtain a uniform coating around the spherical outer surface of the precursor without overheating the polymeric substrate. A coating thickness in the range of 300 Å to 1 μm has little measurable effect upon overall density and is suitable. A suitable magnetron rf sputtering unit is manufactured by Sloan Manufacturing Co. and designated Model No. S310. Another coating technique which may be employed is chemical vapor deposition.

The polymer beads are placed in a shallow aluminum cup or container affixed to a platform inside the sputtering system. Preferably, the beads are introduced through a vacuum interlock in order to minimize pumpdown time. The sputtering system consists of a vacuum chamber which houses the sputtering source and which must be evacuated to a pressure $\leq 3 \times 10^{-6}$ Torr before deposition can be started; higher pressures will lead to films of poor quality which is exhibited primarily by the inability of the coated shells to withstand steam autoclaving. After reaching the required base pressure the system is backfilled with the sputtering gas (argon) to a pressure of ~ 10 mTorr. Using the Sloan Model S310 sputtering system, the shallow container which holds the beads has been 7.5 to 10 cm in diameter.

Prior to and during deposition, the platform to which the cup is affixed is vibrated in a horizontal plane. Vibrating the platform bounces and rotates the polymer beads randomly, which allows the beads to be coated uniformly. A uniform coating is generally necessary if a thin coating is to survive steam autoclaving. It has been found that coating uniformity for beads of a given size was sensitive to vibrational frequency and amplitude. For polymer beads with diameters in the range 100 μm to 200 μm , optimal vibration frequency and amplitude was 37 Hz and ~ 1.5 mm

respectively.

To obtain maximum throughout, it is desirable to maximize the sputter deposition rate. The maximum deposition rate which can be achieved will depend primarily on the maximum temperature to which the substrate can be heated without degrading the surfaces of the polymer beads. It has been found that a rate of .05 $\mu\text{m/hr}$ will yield a smooth coating from a Pyrex® or silica target on polystyrene beads with diameters of ~ 150 μm . For the particular rf sputtering system described, this rate was obtained at an argon pressure of 10 mTorr and an operating power of 0.75 kW. To obtain complete surface coverage, a minimum coating thickness of $\sim .03$ μm is required. To reuse the glass coated polymer beads, they must be capable of being rewashed and reautoclaved. A coating thickness of 0.1 μm has been found to survive a minimum of 2 cycles of rewashing and reautoclaving, and still support active growth of KB cells, MRC-5 cells, Walker 256 carcinosarcoma cells and Murine fibrosarcoma cells.

The microcarriers of the present invention to the extent hereinabove described embody a number of significant advantages. Foremost is the fact that such microcarriers may be fabricated much less expensively than is the case with the all-glass microcarriers of the above-referenced copending application, while retaining the advantages which inhere in a glass surface for cell anchorage and an overall density which is suspendable in the culture medium — i.e. has neutral buoyancy in the selected medium. Additionally, the preferred rf sputter coating technique allows the glass composition to be readily varied, although high silica or pure silica glass are presently envisioned for most cell culturation applications. The polymeric spherical substrate with the basic density of about 1 g/cc may, of course, be manufactured (or purchased) very inexpensively in a variety of sizes.

In accordance with a second important aspect of the present invention, an intermediate layer of magnetic material, such as a nickel coating, may be deposited on the polymeric substrate prior to deposition of the outer silicate glass layer. This is preferably accomplished in an rf sputtering operation as previously described. The magnetic material layer does not have any substantial effect upon microcarrier density. The microcarriers may then be readily removed from suspension in a culture medium, such as by insertion of a permanent magnet into the medium. In accordance with this aspect of the invention in its broadest aspects, the magnetic material may be otherwise included in the microcarrier, such as by occlusion within the polymeric substrate.

CLAIMS

1. A microcarrier adapted for use as growth cites for anchorage dependent cells in an aqueous cell culture medium comprising a spherical substrate of polymeric material having a density of about 1 g/cc and a thin coating of silicate glass

surrounding said substrate.

2. The microcarrier set forth in claim 1 wherein said substrate has a diameter in the range of 50 μm to 500 μm and a density in the range of 1.00 g/cc to 1.10 g/cc, and wherein said coating

3. The microcarrier set forth in claim 1 or 2 comprising a coating of magnetic material surrounding said substrate beneath said silicate glass coating.

4. A method of fabricating a microcarrier for anchorage-dependent cell culturation comprising the steps of:

(a) selecting a spherical precursor of polymeric material having a density of about 1 g/cc, and

(b) coating said precursor with a layer of silicate glass.

5. The method set forth in claim 4 wherein said step (b) is accomplished in an rf sputtering operation.

6. The method set forth in claim 4 or 5 comprising the additional step prior to said step (b) of:

(c) coating said precursor with a layer of magnetic material, said layer of silicate glass being placed over said layer of magnetic material.

7. The method set forth in claim 6 wherein said step (c) is accomplished in an rf sputtering operation.

8. In a microcarrier adapted for use as growth cites for anchorage dependent cell culturation in aqueous media, the improvement wherein material composition of said microcarriers consist in part of magnetic material.